

**Amendments to the Claims:**

*This listing of claims will replace all prior versions and listings of claims in the application.*

**Listing of Claims:**

1. (Withdrawn-Currently Amended) An assay for determining the level of prostacyclin in plasma comprising:

(1) providing a plasma sample;

(2) incubating the plasma sample with an effective amount of an anti-6-keto-prostaglandin  $F_{1\alpha}$  (6-keto-PGF $_{1\alpha}$ ) antibody; an anti-immunoglobulin antibody that binds to the anti-6-keto-PGF $_{1\alpha}$ -antibody; and a conjugate comprising 6-keto-PGF $_{1\alpha}$  covalently bound to an aequorin mutant;

wherein said aequorin mutant comprises serine substitutions for all three cysteine residues as present in wild-type aequorin (Cys  $\rightarrow$  Ser), wherein said aequorin mutant further comprises a single cysteine residue substituted at amino acid position 69 (Ala69  $\rightarrow$  Cys), 70 (Gly70  $\rightarrow$  Cys), 74 (Gly74  $\rightarrow$  Cys) or 76 (Glu76  $\rightarrow$  Cys), and

wherein the 6-keto-PGF $_{1\alpha}$  is coupled to the aequorin mutant via reaction with the sulfhydryl group of the single cysteine;

(3) removing any unbound anti-6-keto-PGF $_{1\alpha}$ -antibody and said conjugate from the plasma sample following incubation; and

(4) measuring and correlating light intensity of the plasma sample with amount of prostacyclin within the plasma sample.

2. (Withdrawn-Previously Presented) The assay of claim 1 wherein the anti-immunoglobulin antibody that binds to the anti-6-keto-PGF $_{1\alpha}$ -antibody is coated onto a surface which is exposed to the plasma, anti-6-keto-PGF $_{1\alpha}$ -antibody and said conjugate.

3. (Cancelled).

4. (Withdrawn) The assay of claim 1 wherein the plasma sample is obtained from a patient receiving intravenous prostaglandin therapy.

5. (Withdrawn-Previously Presented) The assay of claim 1 wherein the concentration of said conjugate in the assay is about  $1 \times 10^{-10}$  M.

6. (Cancelled).

7. (Cancelled).

8. (Withdrawn-Currently Amended) A method of determining an appropriate dose of prostaglandin for the treatment of primary pulmonary hypertension in a patient comprising

(1) providing a plasma sample from the patient;

(2) incubating the plasma sample with an effective amount of;

an anti-6-keto-prostaglandin  $F_{1\alpha}$  (6-keto-PGF $_{1\alpha}$ ) antibody; an anti-immunoglobulin antibody that binds to the anti-6-keto-PGF $_{1\alpha}$ -antibody; and a conjugate comprising 6-keto-PGF $_{1\alpha}$  covalently bound to an aequorin mutant;

wherein said aequorin mutant comprises serine substitutions for all three cysteine residues as present in wild-type aequorin (Cys  $\rightarrow$  Ser), wherein said aequorin mutant further comprises a single cysteine residue substituted at amino acid position 69 (Ala69  $\rightarrow$  Cys), 70 (Gly70  $\rightarrow$  Cys), 74 (Gly74  $\rightarrow$  Cys) or 76 (Glu76  $\rightarrow$  Cys), and

wherein the 6-keto-PGF $_{1\alpha}$  is coupled to the aequorin mutant via reaction with the sulfhydryl group of the single cysteine,

(3) removing any unbound anti-6-keto-PGF $_{1\alpha}$ -antibody and said conjugate from the plasma sample following incubation;

(4) measuring and correlating the amount of detected 6-keto- PGF $_{1\alpha}$  with the appropriate dosage of prostaglandin for the patient.

9. (Withdrawn-Previously Presented) The method of claim 8 wherein the anti-immunoglobulin antibody is coated onto a surface which is exposed to the plasma, anti-6-keto-PGF $_{1\alpha}$ - antibody and said conjugate.

10. (Cancelled).

11. (Withdrawn) The assay of claim 8 wherein the plasma sample is obtained from a patient receiving intravenous prostaglandin therapy.

12. (Withdrawn-Previously Presented) The assay of claim 8 wherein the concentration of said conjugate in the assay is about  $1 \times 10^{-10}$  M.

13. (Withdrawn-Currently Amended) An assay for determining the level of a biomolecule in plasma comprising:

- (1) providing a plasma sample;
- (2) incubating the plasma sample with an effective amount of an anti-6-keto-prostaglandin  $F_{1\alpha}$  (6-keto-PGF $_{1\alpha}$ ) antibody to the biomolecule, an anti-immunoglobulin antibody that binds to the biomolecule and a biomolecule-aequorin conjugate comprising 6-keto-PGF $_{1\alpha}$  covalently bound to an aequorin mutant;

wherein said aequorin mutant comprises serine substitutions for all three cysteine residues as present in wild-type aequorin (Cys  $\rightarrow$  Ser), wherein said aequorin mutant further comprises a single cysteine residue substituted at amino acid position 69 (Ala69  $\rightarrow$  Cys), 70 (Gly70  $\rightarrow$  Cys), 74 (Gly74  $\rightarrow$  Cys) or 76 (Glu76  $\rightarrow$  Cys), and

wherein the 6-keto-PGF $_{1\alpha}$  is coupled to the aequorin mutant via reaction with the sulfhydryl group of the single cysteine;

- (3) removing any unbound anti-6-keto-PGF $_{1\alpha}$  antibody and biomolecule-aequorin conjugate from the plasma sample following incubation; and

- (4) measuring and correlating light intensity of the plasma sample with amount of biomolecule within the plasma sample.

14. (Withdrawn-Previously Presented) The assay of claim 13 wherein the anti-immunoglobulin antibody is coated onto a surface which is exposed to the plasma, anti-6-keto-PGF $_{1\alpha}$  antibody and biomolecule-aequorin conjugate.

15-18. (Cancelled).

19. (Withdrawn) The biomolecule aequorin conjugate of claim 17 wherein the biomolecule is a peptide.

20. (Withdrawn-Currently Amended) A method for determining the effect of a therapeutic agent on the level of prostacyclin in the plasma of a patient comprising

- (1) administering the therapeutic agent to the patient;
- (2) obtaining a plasma sample from the patient;
- (3) incubating the plasma sample with an effective amount of;

an anti-6-keto-prostaglandin  $F_{1\alpha}$  (6-keto-PGF $_{1\alpha}$ ) antibody; an anti-immunoglobulin antibody that binds to the anti-6-keto-PGF $_{1\alpha}$ -antibody; and a conjugate comprising 6-keto-PGF $_{1\alpha}$  covalently bound to an aequorin mutant;

wherein said aequorin mutant comprises serine substitutions for all three cysteine residues as present in wild-type aequorin (Cys  $\rightarrow$  Ser), wherein said aequorin mutant further comprises a single cysteine residue substituted at amino acid position 69 (Ala69  $\rightarrow$  Cys), 70 (Gly70  $\rightarrow$  Cys), 74 (Gly74  $\rightarrow$  Cys) or 76 (Glu76  $\rightarrow$  Cys), and

wherein the 6-keto-PGF $_{1\alpha}$  is coupled to the aequorin mutant via reaction with the sulfhydryl group of the single cysteine;

(4) removing any unbound anti-6-keto-PGF $_{1\alpha}$  antibody and said conjugate from the plasma sample following incubation; and

(5) measuring and correlating light intensity of the plasma sample with amount of prostacyclin within the plasma sample.

21. (Cancelled).

22. (Currently Amended) A kit for measuring prostacyclin in plasma comprising:

- (1) an anti-6-keto-prostaglandin  $F_{1\alpha}$  (6-keto-PGF $_{1\alpha}$ ) antibody;
- (2) an anti-immunoglobulin antibody that binds to the anti-6-keto-PGF $_{1\alpha}$ -antibody; and

(3) a conjugate comprising 6-keto-PGF $_{1\alpha}$  covalently bound to an aequorin mutant; wherein said aequorin mutant comprises serine substitutions for all three cysteine residues as present in wild-type aequorin (Cys  $\rightarrow$  Ser), wherein said aequorin mutant further comprises a

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single cysteine residue substituted at amino acid position 69 (Ala69 → Cys), 70 (Gly70 → Cys), 74 (Gly74 → Cys) or 76 (Glu76 → Cys), and wherein the 6-keto-PGF<sub>1α</sub> is coupled to the acquorin mutant via reaction with the sulfhydryl group of the single cysteine.